

## Effects of aerosolized endotoxin in feedyard dust on weanling goats<sup>☆</sup>

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### Abstract

Weanling female Spanish goats ( $n = 36$ ) were randomly allotted to four treatment groups (each group,  $n = 9$ ): principal dust group with antibiotic; principal dust group without antibiotic; control group with antibiotic; and control group without antibiotic. The principals were exposed to twenty-two 4 h dust treatments in a closed tent. Dust treatments occurred as follows: one dust treatment, 18-day rest, seven daily dust treatments, 7-day rest, seven daily dust treatments, 7-day rest, and seven daily dust treatments. All principals and six controls were euthanatized and necropsied at the end of the experiment. There was an interaction between dust and tilmicosin antibiotic for feed intake and average daily gain following the first dust treatment. The interaction occurred because control goats not exposed to dust nor receiving tilmicosin protection had lower ( $P < 0.05$ ) feed intake than those principals exposed to dust without tilmicosin protection (996 g per head per day versus 1131 g per head per day, respectively). Consequently, these goats also gained more ( $P < 0.02$ ) weight than the control goats without tilmicosin protection (57 g per head per day versus 42 g per head per day). Contrary to the hypothesis that dust might reduce the efficiency of feed utilization, it tended to improve it. After the first dust treatment, the mean rectal temperatures of the four groups with and without antibiotics were significantly different ( $P \leq 0.0003$ ). Four hours ( $P \leq 0.0016$ ) after dust treatment, the mean rectal temperature of the two principal groups was higher than the controls. On the first 7-day dust treatment series there was a significant ( $P \leq 0.0006$ ) difference between the groups. The mean rectal temperatures of the two principal groups at 8 h on day 1 were significantly ( $P \leq 0.02$ ) higher than the control groups. On the second 7-day dust treatments, there were no significant differences in mean rectal temperature between the groups. On the third 7-day treatment series there were significant ( $P \leq 0.0001$ ) differences among the four groups. On the first day of the dust treatment series, at 8 h, the trend of the principal groups' mean rectal temperature was higher than the controls. It appears that repeated endotoxin/dust exposures induces a state of tolerance for increased rectal temperatures that are characteristic after one dust exposure. There were no gross observable differences between the controls and principal respiratory tracts. The following histologic changes were observed in the principal goats: a mild alveolar septal hypercellularity, moderate multifocal airway exudation of neutrophils

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and macrophages containing foreign particulate material, and there was a marked increase in bronchial associated lymphoid tissue compared to controls. The diagnosis was mild acute exudative bronchointerstitial pneumonia. No significant microscopic lesions were observed in the control goats.

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## 1. Introduction

Air quality has become a pressing issue for public health, first in urban communities and later in rural communities. The Clean Air Act Amendments (CAAA) of the 1970s required the US Environmental Protection Agency (EPA) to set national ambient air quality standards (NAAQSs) to protect the environment and its human inhabitants. In 1977, the EPA was given the enforcement powers and ability to fine any industry which exceeds the maximum permissible level of particulate matter in the air. This level was based on a maximum or aerodynamic diameter of  $10\text{ }\mu\text{m}$  or less size particles ( $\text{PM}_{10}$ ) per year not to exceed more than once, average  $50\text{ }\mu\text{g}/\text{m}^3$ , or per 24 h not to exceed  $150\text{ }\mu\text{g}/\text{m}^3$ . In 1997, this level was changed to a  $\text{PM}_{2.5}$  or aerodynamic diameter of  $2.5\text{ }\mu\text{m}$  or less size particles, per year not to exceed more than once, average  $15\text{ }\mu\text{g}/\text{m}^3$ , or per 24 h not to exceed  $50\text{ }\mu\text{g}/\text{m}^3$  (Marquardt, 1998). Concentrations of particles in the air vary with temperature and pressure and the EPA has selected these to be measured at  $25\text{ }^\circ\text{C}$  and  $760\text{ mmHg}$  (Marquardt, 1998). The smaller particles are considered more dangerous to the health of humans or animals, because they can be inhaled much deeper into the respiratory tract (Schwartz, 1997). Particles of  $1\text{ }\mu\text{m}$  in diameter or less can be inhaled into the alveolar sacs of the lungs. Urban air pollution has become a worldwide health hazard, because industrialization and car ownership continues to spread (McMichael, 1997).

Animal production of any kind introduces manure into the environment. High animal concentrations produce large volumes of manure. Examples include large integrated chicken farms (Clark et al., 1983) in the southeastern US, and hog farms (Zejda et al., 1993; Holness et al., 1987) in the Oklahoma and Texas panhandle area. The feeder calf industry in the Southern High Plains may stock 100,000 calves in one feedyard per year, which results in 100,000 t

of dry manure. When manure dries it produces dust particles (Sweeten et al., 1988), high in endotoxins originating from Gram-negative bacteria. The literature was searched for the effects of endotoxin/dust administered as an aerosol to ruminants, but none was found. Therefore, we searched the human literature to see what effects endotoxin induced in human subjects.

Clinically, endotoxin (doses  $20\text{--}300\text{ }\mu\text{g}$ ) via the respiratory route in humans commonly induces tightness of the chest, airway irritation, and fever after 6–8 h. Less common symptoms are headache, joint and muscle pains, nausea, and fatigue (Rylander et al., 1989). Chest tightness, cough, dyspnea, and sputum production were reported after the inhalation of a  $0.9\text{ }\mu\text{g}/\text{ml}$  endotoxin dose (Jagiello et al., 1996). An increase in peripheral total leukocyte counts and neutrophils was observed 6 h after inhalation of endotoxin ( $30\text{--}60\text{ }\mu\text{g}$  in a delivery of  $4.5\text{--}8.1\text{ ml}$  Hanks balanced salt solution) in humans (Clapp et al., 1993).

The objectives of this study were to determine the clinical effects, clinical pathology, and histopathologic effects of aerosolized feedyard endotoxin/dust on weanling principal goats treated in a semi-airtight tent compared to non-dust treated weanling controls. The effects of an antibiotic were also tested on the principal dust and control groups to determine if it afforded any protective effect to those goats that received dust treatments. Several aerosol devices and techniques were used to analyze the feedyard dust administered to the goats. The treatment dust was analyzed for its size, amount, microbial and endotoxin content. All animal studies were approved by the regional animal care committee.

### 1.1. Hypothesis

Goats exposed to dust treatments will have reduced feed intake, reduced average daily weight gain, and increased feed/gain ratios.

## 1.2. Objectives

The four treatment groups were compared to each other by measuring their rectal temperature response, daily food consumption and feed efficiency, and weekly weight response. The total complete blood cell (CBC) counts, fibrinogen and haptoglobin concentrations were compared between the principal goat dust group without antibiotics and the control goat group without antibiotics prior to treatment, and after the first dust treatment and prior to and after the first series of seven daily dust treatments.

## 2. Materials and methods

### 2.1. Goats

Thirty-six Spanish weanling female goats were housed in a three-sided barn. They were treated for internal helminth parasites (Ivomec, MSD AGVET, Merck, Rahway, NJ) and coccidia (Amprolium, MSD AGVET, Merck, Rahway, NJ). Goats were limit-fed a commercial pelletized ration (44% grain concentrate, 20% alfalfa hay, 30% cottonseed hulls and meal, 5% molasses, Vitamins A and E, and trace minerals) and watered free choice.

### 2.2. Antibiotic

Trade name Micotil 300, chemical name tilmicosin, administered subcutaneously at a dose of 1 ml/30 kg body weight, Elanco Animal Health, Eli Lilly & Co., Indianapolis, IN).

### 2.3. Experimental design

The goats were randomly allotted to 12 pens (7420 cm<sup>2</sup>), three goats per pen. The goats were randomized into four treatment groups (each group,  $n = 9$ ): principal dust goats with tilmicosin; principal dust goats without antibiotics; control goats with tilmicosin; and control goats without antibiotic. The goats were handled for 2 weeks prior to the start of the experiment to accustom them to the feeding, the taking of rectal temperatures, and to jugular blood sampling.

All goats had their rectal temperature taken prior to dust treatment and after one dust treatment (at

4, 8, 12, 24 and 48 h). Total WBC counts and differentials (including fibrinogen (Millar et al., 1971) and haptoglobin (Roy et al., 1969)) were taken prior to and after one dust treatment (at 4, 8, 12, 24 and 48 h) on the principal goats without antibiotics and on the control goats without antibiotics. Eighteen days after the first dust treatment, a series of seven daily dust treatments were administered, and after that the goats were rested for 1 week. The same process was repeated two more times.

Prior to the initiation of the study, goats were fed a standard growing diet and feed intake was measured daily for 7 days to estimate maximum feed intake. During the study, goats had access to feed and water ad libitum. Daily feed intake and refusals were measured during the study and the goats were weighed every 7 days. Feed intake, average daily weight gain (ADG) and feed/gain ratios were calculated. All the data were subjected to the analysis of variance using the General Linear Models procedure of SAS (1996). The model for the performance data analysis included the main effects of dust, tilmicosin and their interactions. The experimental unit was the pen (three goats per pen) and the error term was the interaction where the interaction was significant; otherwise the experimental error term was used for testing of significance between treatment means. Mean comparisons were performed by the least square means procedure of SAS (1996).

Three dust principal goats without antibiotic and three dust principal goats with tilmicosin were necropsied 8 h following final dust treatment. Two non-dust control without antibiotics goats were necropsied. The remaining 12 principal goats (6 plus tilmicosin and 6 minus antibiotic) were necropsied 4 days after the last dust treatment. The goats were taken to the Veterinary Medical Diagnostic Laboratory, Texas A&M University, Amarillo, TX, for euthanasia and necropsy. The gross and histopathologic examination of the tissues were determined on the dust principals and the non-dust controls without the pathologist knowing their group status.

### 2.4. Sampling intervals

The rectal temperature and total complete blood cell count were measured following a time series relative to dust treatment: pre-dust treatment, 4 h dust treat-

ment, 4, 8, 12, 24 and 48 h post-dust treatment. The time series was modified for collection of blood for white blood cell (WBC) counts when seven consecutive dust treatments were administered. The data were similarly collected on day 1, then lapsed to day 7 when the blood sample was again taken prior to the 4 h dust treatment, and at 4, 8, 12, 24 and 48 h post-dust treatment.

## 2.5. Tents

The semi-airtight dust tent (183 cm wide  $\times$  244 cm long  $\times$  213 cm tall) was used to contain the administered dust and the goats, it was previously described (Purdy et al., 2002a,b).

## 2.6. Feedyard dust preparation

Dried feedyard manure was removed from a working feedyard, processed to a fine dust (Purdy et al., 2002a,b), and was stored in plastic barrels. A sufficient quantity of processed dust was stored and used as needed to conduct the study.

## 2.7. Microbial culture and endotoxin assay of dust

Microbial activity (bacterial and fungal) was determined, using standard microbial dilutions techniques. Anaerobic, aerobic, and thermophilic bacteria, and mesophilic and thermophilic fungi were cultured as described in (Purdy et al., 2002a,b).

A second method was used to quantify and size viable microbes contained in the dust as an aerosol inside the tent. This method was executed by using three six-stage Andersen biological cascade impactors (Andersen Sampler Inc., Atlanta, GA) fitted with 100 mm  $\times$  15 mm Petri dishes containing appropriate media. The Andersen impactors were placed 120 cm above the floor and calibrated vacuum pumps (1 ft<sup>3</sup>/min) were turned on for 30 s to sample for bacteria, and 90 s to sample for fungi. The three Andersen biological impactors were used to determine the mean quantity of microbes on each medium as described above for the thermophilic bacteria, mesophilic bacteria and anaerobic bacteria, and the same was completed for thermophilic fungi, and mesophilic fungi. The microbes were quantified by

number of colonies that grew on each plate and the microbes size or size of particle that the microbe was traveling on, was estimated by the size of the hole in each plate (each stage has 400 jet holes). Diameters of the jet holes (cm) were as follows: stage 1, 0.118; stage 2, 0.091; stage 3, 0.071; stage 4, 0.053; stage 5, 0.034; stage 6, 0.025. Stages 5 and 6 gathers particles sufficiently small that they could reach deep into the lung alveoli on the inspiration of an animal. Andersen cascade samplers simulate the human respiratory system (Andersen Operating Manual for Viable Microbial Particle Sizing Sampler, TR#76-900042, Atlanta, GA, 1976, 8 pp.). For example, stage 1 collects 7  $\mu$ m particles which can enter the nose, stage 2 collects 4.7–7  $\mu$ m particles which can enter the pharynx, stage 3 collects 3.3–4.7  $\mu$ m particles which can enter the trachea and primary bronchi, stage 4 collects 2.1–3.3  $\mu$ m particles which enter the secondary bronchi, stage 5 collects 1.1–2.1  $\mu$ m particles which enter the terminal bronchi, and stage 6 collects 0.065–1.1  $\mu$ m particles which can enter the alveoli.

The dust aerosol was also analyzed for endotoxin concentration by collecting dust for 30 min with three Andersen two-stage microbial cascade impactors. Two glass Petri dish bottoms each containing 20 ml of sterile reverse osmosis (RO) water were carefully placed in the bottom stage 6 and the top stage 1 (with stages 2–5 removed) of a six-stage Andersen microbial cascade impactor. (*Note:* only the pegs on the six-stage Andersen impactor are small enough to accommodate the thick glass plates.) In addition, a series of three glass bottle traps each containing 20 ml of RO water were hooked into the impactor to determine the amount of endotoxin that might go through the impactor and be lost to the environment. After endotoxin collection the water from the three traps was combined into one sample. The samples analyzed for endotoxin concentration were the water from stage 1 (non-respirable particles), stage 6 (respirable particles) and the water from the traps (respirable particles). For the sake of reporting, stage 1 was called 0, and stage 6 was called 00. The Andersen impactors were placed 120 cm above the floor.

Finally the feedyard dust was also analyzed by the multiple tube method for Gram-negative microbes described in detail (Purdy et al., 2002a,b).

## 2.8. Challenge technique

The dust aerosol technique used to challenge the goats is described in detail (Purdy et al., 2002b) in a companion paper.

## 2.9. Endotoxin assay

The endotoxin/dust was measured using the kinetic chromogenic semi-quantitative Limulus amoebocyte lysate assay. This assay is described (Purdy et al., 2002b) in a companion paper.

## 2.10. Determination of dust particle size

Three Andersen six-stage biological cascade impactors were used to determine the amount of viable microbes (bacteria and fungi) and the size of the microbe or size of the particle on which the microbe was traveling. The six-stage cascade impactor was set for 28.3 l/min, and it was run for 30 s to determine the viable bacterial colonies and for three min to determine the viable fungal colonies. Three two-stage Andersen cascade impactors were used to collect endotoxin. The two-stage Andersen impactors were run for 30 min at the rate of 28.3 l air/min.

A five-stage cyclone device (In-Tox Products, Albuquerque, NM) was used to quantify the small particles of dust  $<0.32 \mu\text{m}$  which collected on the filter paper. Stage 5 measured particles of  $0.32 \mu\text{m}$ , stage 4 particles of  $0.65 \mu\text{m}$ , stage 3 particles of  $1.4 \mu\text{m}$ , stage 2 particles of  $2.1 \mu\text{m}$ , and stage 1 particles of  $5.4 \mu\text{m}$ .

Eight Millipore Swinnex holders (Swinnex filter holders, Millipore Corp., Bedford, MA) (47 mm) were equipped with  $0.45 \mu\text{m}$  filters. These filters were used to determine the amount of dust in the air of the dust tent. Andersen vacuum pumps were used to collect 28.3 l air/min. All gravimetric filter determinations were done in duplicate. The filters were pre-weighed and after 30 min weighed again to determine the quantity of dust/ $\text{m}^3$  in the tent during the dust treatment. The duplicate samples were averaged.

Five open Petri dishes were placed on the board which supported the cyclone device. These dishes were allowed to collect dust particles which settled out of the air during the 4 h period. These dishes were weighed before and after collection of the dust particles.

A Malvern Mastersizer 2000 particle size analyzer (Malvern Instruments Inc., Southborough, ME) with optical bench (dual 466 nm blue LED and 2 mW/633 nm HeNe laser light source) was used to size and quantify the range of feedyard dust particles. These readings were performed in triplicate.

### 2.10.1. Statistical analysis

The mean results for measured variables were compared by analysis of variance using the General Linear Models procedure of SAS (1996). Means of rectal temperature, total WBC counts, absolute neutrophil and lymphocyte counts, and fibrinogen and haptoglobin concentrations were compared between principal and control groups over the experiment, and within any sample day period of time. Significant differences between treatment means were determined by Bonferroni's and Dunnett's adjusted paired *t*-test ( $P \leq 0.05$ ) that allowed pairwise comparisons of treatment group means and control means within any sample collection day. The multiple tube method was used to determine the total coliforms, total fecal coliforms, and *Enterococcus faecalis* contained in the feedyard dust. These were calculated to the 50% endpoint by the method of Reed and Muench (1938).

## 3. Results

### 3.1. Endotoxin in dust aerosol

Two-stage Andersen impactors ( $n = 3$ ) collected the following concentrations (ng) of endotoxin per ml over 30 min. Number 1 impactor: non-respirable plate 0, 97.0; respirable plate 00, 700.0; combined traps, 25.1. Number 2 impactor: non-respirable plate 0, 65.9; respirable plate 00, 700.0; combined traps, 45.6. Number 3 impactor: non-respirable plate 0, 35.1; respirable plate 00, 224.0; combined traps, 9.2. Each unit contained 20 ml, therefore, each datum point was multiplied by a factor of 20 for total concentration collected over 30 min. The mean endotoxin concentration of non-respirable plate 0, was 1320.0 ng/20 ml (S.E.M. 619), respirable plate 00, was 7760.0 ng/20 ml (S.E.M. 5406), and combined traps ( $n = 9$ ), was 533.0 ng/20 ml (S.E.M. 365). The mean total respirable endotoxin (respirable plate 7760.0 ng/20 ml



plus mean combined trap endotoxin 533.0 ng/20 ml) equaled 8293.0 ng over 30 min (pump 28.3 l/min).

### 3.2. Analyses of feedyard dust

Feedyard dust contained 26.9 µg of endotoxin/g according to the IBT Reference Laboratory analysis. The mean viable microbial colony counts/g dust was determined by a series of 10-fold dilutions each of which was cultured on the appropriate medium with the following results: anaerobic bacteria,  $6.3 \times 10^7$  (S.E.M.  $2.4 \times 10^7$ ); mesophilic bacteria,  $1.5 \times 10^8$  (S.E.M.  $1.9 \times 10^7$ ); thermophilic bacteria  $6.3 \times 10^5$  (S.E.M.  $3.3 \times 10^6$ ); mesophilic fungi  $1.4 \times 10^4$  (S.E.M.  $1.0 \times 10^5$ ); and thermophilic fungi  $1.3 \times 10^3$  (S.E.M.  $4.5 \times 10^2$ ).

Andersen six-stage viable microbial cascade impactor ( $n = 3$ ) stages 1–6 colonies were each cultured and quantified as mean bacterial and fungal colony forming unit (CFU) per culture plate in each stage (Table 1). No attempt was made to identify the bacteria from the Andersen impactors. The most numerous mesophilic fungi on both the malt agar and LOA were *Penicillium* spp. On LOA media (stage 1) there were a total (all three impactors) of 222 CFU and of these 45% were *Penicillium* spp.; stage 2, total of 160 CFU and of these 93% were *Penicillium* spp.; stage 3, total of 251 CFU and of these 91% were *Penicillium* spp.; stage 4, total of 518 CFU and of these 90% were *Penicillium* spp.; stage 5, total of 214 CFU and

of these 72% were *Penicillium* spp.; and stage 6, total of 2111 CFU and of these 97% were *Penicillium* spp. Other fungi identified on LOA from feedyard dust were sterile mycelia (32 colonies) on plates from stage 1, *Cladosporium* spp. on plates from stage 1 (10 colonies) and stage 3 (11 colonies), and *Mucor* spp. on plates from stage 4 (28 colonies) and stage 5 (15 colonies). Other less numerous fungi found on LOA were *Gliocladium*, *Fusarium*, *Scopulariopsis*, *Aspergillus*, and *Alternaria*. On malt medium there was a total (all three impactors) of 161 CFU in stage 1 and of these 83% were *Penicillium* spp.; stage 2, total of 121 CFU and of these 95% was *Penicillium* spp.; stage 3, total of 656 CFU and of these 60% were *Penicillium* spp.; stage 4, total of 781 CFU and of these 56% were *Penicillium* spp.; stage 5, total of 605 CFU and of these 75% were *Penicillium* spp. and stage 6, total of 186 CFU and of these 93% were *Penicillium* spp. Other less numerous fungi found on malt agar were *Alternaria*, *Mucor*, *Aspergillus*, *Bipolaris*, *Cladosporium*, *Fusarium*, and sterile mycelia. Of the thermal fungi, 11 colonies were identified as *Penicillium duponti*, and 2 colonies were identified as *Montospora lanuginosa*.

The Andersen distribution of total small size particles recovered as viable colonies was determined for stages 4–6 from the Andersen data presented (Table 3). For example, the mesophilic mean total bacterial colony count for stages 4–6 was 1871 viable colonies which was 46% of the mean total count (4084) for

Table 1

Mean culture results in bacterial (30 s run at 1 ft<sup>3</sup>/min of vacuum) and fungal colony forming units (3 min run at 1 ft<sup>3</sup>/min of vacuum) of three Andersen six-stage cascade impactors

Stage	Size of 400 jet holes (cm)	Mean (CFU)					
		Anaerobic bacteria	Mesophilic bacteria	Thermophilic bacteria	Mesophilic fungi (malt)	Mesophilic fungi (LOA)	Thermophilic fungi (malt)
1	0.118	250 (3)	833 (173)	198 (21)	54 (13)	74 (19)	4
2	0.091	381 (31)	620 (147)	99 (16)	40 (22)	53 (18)	0
3	0.071	368 (40)	760 (223)	157 (36)	219 (69)	84 (54)	0
4	0.053	441 (66)	680 (40)	409 (21)	260 (58)	173 (57)	5
5	0.034	349 (91)	491 (29)	415 (47)	202 (45)	71 (14)	4
6	0.025	272 (12)	700 (171)	292 (22)	59 (21)	724 (550)	0
Mean total	N.A.	2061	4084	1570	834	1179	13

Each stage has 400 holes through which microbes and particles flow through depending on size of microbes and particles. Numbers in parentheses indicate S.E.M. Bacteria were grown on brain heart infusion agar. Fungi were grown on malt agar and Littman oxgall agar (LOA). Grand mean total of all bacterial colonies cultured from the aerosol in the tent for 30 s was 7715 CFU, dust treatment 4 h. Grand mean total of all fungal colonies cultured from the aerosol in the tent for 3 min was 2026 CFU, dust treatment 4 h.

stages 1–6. The thermophilic mean total bacterial colony count for stages 4–6 was 1116 viable colonies which was 71% of the mean total 1570 thermophilic bacterial colony count for stages 1–6. It can be concluded from the Andersen distribution of small respiratory particles that many viable particles are being inhaled deeply into the goat lungs.

### 3.3. Microbial analysis of treatment dust

The mean total coliforms per gram of dust was  $8.5 \times 10^5$  (S.E.M.  $7.0 \times 10^5$ ), mean total fecal coliforms was  $6.8 \times 10^4$  (S.E.M. 0.0), and mean total *E. faecalis* was  $3.2 \times 10^4$  (S.E.M.  $3.2 \times 10^4$ ). *Salmonella* spp. were not isolated from the feedyard dust.

### 3.4. Malvern Mastersizer 2000 histogram report

The feedyard dust was analyzed dry by passing 1 g samples through the analyzer three times. The three histograms were very similar to each other. The particle sizes ranged from 0.89 to 355.6  $\mu\text{m}$  with a mean of 100.027  $\mu\text{m}$ . The particle size distribution was as follows: 0.89–1.002  $\mu\text{m}$ , 0.04 volume in percentage; 1.416–1.689  $\mu\text{m}$ , 0.10 volume in percentage; and 10.00–11.247  $\mu\text{m}$ , 0.42 volume in percentage.

### 3.5. Millipore filter collection of feedyard dust from inside of tent during 4 h dust treatment

The mean dust collected from the tent was: 0–0.5 h, 49.628  $\text{mg}/\text{m}^3$  (S.E.M. 4.92); 0.5–1.0 h, 46.869  $\text{mg}/\text{m}^3$  (S.E.M. 3.90); 1.0–1.5 h, 33.477  $\text{mg}/\text{m}^3$  (S.E.M. 2.86); 1.5–2.0 h, 47.877  $\text{mg}/\text{m}^3$  (S.E.M. 3.45); 2.0–2.5 h, 50.038  $\text{mg}/\text{m}^3$  (S.E.M. 4.36); 2.5–3.0 h, 58.675  $\text{mg}/\text{m}^3$  (S.E.M. 7.34); 3.0–3.5 h, 47.217  $\text{mg}/\text{m}^3$  (S.E.M. 1.91); and 3.5–4.0 h, 70.955  $\text{mg}/\text{m}^3$  (S.E.M. 10.16) (Fig. 1). Grand mean was 50.591  $\text{mg}/\text{m}^3$  (S.E.M. 10.74) (Fig. 1).

### 3.6. Analysis of feedyard dust during dust treatment

The hopper was charged with 1500 g of dust each time a 4 h dust treatment was given to the principal goats. The mean ( $n = 22$ ) for the following parameters were: weight of dust in the trap (464 g, S.E.M. 38), weight of dust that entered the tent over 4 h (843 g, S.E.M. 45), amount of dust in the tent (89  $\text{g}/\text{m}^3$  per 4 h, S.E.M. 5), weight of dust determined for each stage of the cyclone device, stage 1 (0.343 g, S.E.M. 0.042), stage 2 (0.088 g, S.E.M. 0.013), stage 3 (0.092 g, S.E.M. 0.009), stage 4 (0.031 g, S.E.M. 0.003), stage 5

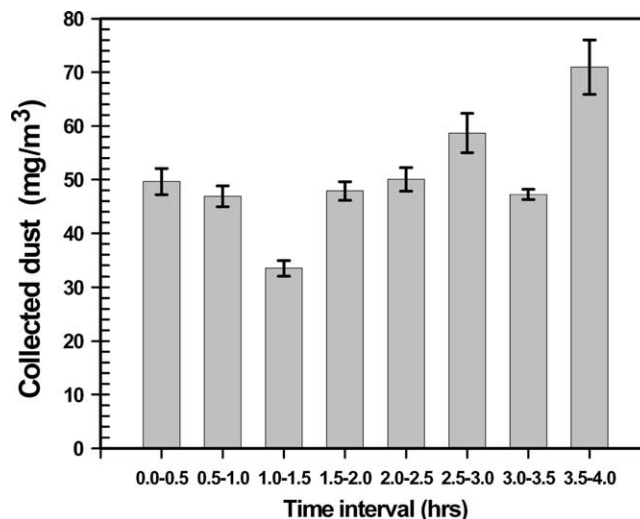


Fig. 1. Inside tent dust aerosol was measured every 30 min by collecting dust onto a filter membrane inside a Millipore device attached by Tygon tubing to an Andersen vacuum pump. Thirty-minute intervals were selected to prevent filter overload. The filters were weighed prior to and after dust collection to determine the amount of dust obtained. S.E.M. is represented by bars.

Table 2  
Dust treatment summary

	Mean ( <i>n</i> = 22)	S.E.M.
Initial dust (g)	1500.0	0.0
Hopper leftover (g)	193.0	21.4
Trap dust (g)	463.7	37.9
Dust in tent (g)	843.3	44.6
Dust in tent (g/(m <sup>3</sup> min))	0.3693	0.0195
Stage 1 (g)	0.3425	0.0423
Stage 2 (g)	0.0880	0.0133
Stage 3 (g)	0.0920	0.0088
Stage 4 (g)	0.00311	0.0031
Stage 5 (g)	0.0256	0.0023
Filter dust (g)	0.0076	0.0008
Dish dust total (g)	5.0121	0.3560
Tent floor dust (g/4.46 m <sup>2</sup> )	734.96	52.20
Tent floor dust (g/m <sup>2</sup> )	164.81	11.71

Stages 1–5 and the filter are the dust collection units of the cyclone device which collects the small particles of dust in the tent. Dish represents the five Petri plates which were used to passively collect the particles which would not stay suspended in the dust aerosol in the tent.

(0.026 g), cyclone filter (0.008 g, S.E.M. 0.0008), weight of large dust particles passively settling into the five Petri dishes (1.002 g, S.E.M. 0.590), weight of large dust particles passively settling over the area of the tent (18.04–28.42 g/m<sup>2</sup>) (Table 2).

### 3.7. Feed intake and average daily gain following the first dust treatment

There was an interaction between dust and tilmicosin antibiotic on feed intake and average daily gain (ADG). The interaction occurred because control goats not exposed to dust nor receiving tilmicosin had lower ( $P < 0.05$ ) feed intake than those principals exposed to dust without tilmicosin (996 g per head per day versus 1131 g per head per day, respectively). Consequently, these goats also gained more ( $P < 0.02$ ) weight than the control goats without tilmicosin (57 g per head per day versus 42 g per head per day). Although, not significant, goats exposed to dust utilized 7.9% less feed per gram of weight gained than those not exposed to dust. This was probably because of the higher rate of gain of these goats. Tilmicosin protected goats utilized 9% less ( $P > 0.05$ ) feed per gram of weight gained than those not protected with antibiotic (19.8 versus 21.8, respectively). Contrary to the hypothesis that dust might reduce the efficiency

Table 3  
Effect of feedyard multiple dust treatments on performance of young Spanish goats

Item	Dust stress		S.E.M. <sup>a</sup>
	No dust <sup>b</sup> ( <i>n</i> = 12)	Dust <sup>c</sup> ( <i>n</i> = 12)	
Initial BW (kg)	21.2	20.9	–
Final BW (kg)	24.7	24.6	–
Feed intake (kg per head per day)	2.83 a	2.92 b	0.10
ADG (g per day)	148.0	163.0	7.03
Feed/gain ratio	19.5 c	17.9 d	0.65

Means within the same row with different letters (a and b) differ ( $P < 0.01$ ). Means within the same row with different letters (c and d) differ ( $P < 0.05$ ). BW = body weight.

<sup>a</sup> Standard error of least square means (*n* = 6).

<sup>b</sup> No dust, no tilmicosin (*n* = 6); no dust, tilmicosin (*n* = 6).

<sup>c</sup> Dust, no tilmicosin (*n* = 6); dust, tilmicosin (*n* = 6).

of feed utilization, it tended to improve it. Tilmicosin administration sustained feed intake and ADG of young Spanish goats. The food consumption of the principals decreased 16.2% by the morning after the first dust treatment compared to the controls, which decreased 12.5%. Five days later the principals were eating a mean of 199 g less than their pre-dust treatment ration, and the controls were eating a mean of 107 g less than their pre-tent treatment ration. Adverse clinical signs were not observed in the controls.

During the entire dust study (first plus multiple dust treatments) there was no interaction between dust and tilmicosin for feed intake, ADG, and feed/gain ratio. The main effect of dust and tilmicosin on animal performance is presented (Tables 3 and 4).

The mean rectal temperatures and WBC counts following one dust treatment for the principal dust without antibiotic compared to the control group without antibiotic are reported. Overall for the 1st dust treatment, the mean rectal temperature of the four groups with and without antibiotic were significantly different ( $P \leq 0.0003$ ). At 4 h ( $P \leq 0.0016$ ) post-dust treatment, the mean rectal temperature of the two principal groups (plus tilmicosin 40.46 °C (S.E.M. 0.158) and minus antibiotic 40.49 °C (S.E.M. 0.133)) was higher than the controls (plus tilmicosin 39.76 °C (S.E.M. 0.018) and minus antibiotic 39.97 °C (S.E.M. 0.177)) (Fig. 2).

For the first 7-day dust treatment series, there was a significant ( $P \leq 0.0006$ ) difference between the



Table 4  
Effect of tilmicosin treatment on performance of young Spanish goats

Item	Antibiotic		S.E.M. <sup>a</sup>
	No tilmicosin <sup>b</sup> (n = 12)	Tilmicosin <sup>c</sup> (n = 12)	
Initial BW (kg)	21.3	20.8	–
Final BW (kg)	24.6	24.8	–
Feed intake (kg per head day)	2.87	2.88	0.10
ADG (g per day)	149.0	162.0	7.03
Feed/gain ratio	19.6 a	17.8 b	0.65

Means within the same row with different letters (a and b) differ ( $P < 0.05$ ). BW = body weight.

<sup>a</sup> Standard error of least square means ( $n = 6$ ).

<sup>b</sup> No dust, no tilmicosin ( $n = 6$ ); dust, no tilmicosin ( $n = 6$ ).

<sup>c</sup> No dust, tilmicosin ( $n = 6$ ); dust, tilmicosin ( $n = 6$ ).

groups. The mean rectal temperature of the principal dust groups at 8 h were significantly ( $P \leq 0.0009$ ) higher (plus tilmicosin  $40.18^\circ\text{C}$  (S.E.M. 0.086) and minus antibiotic  $40.22^\circ\text{C}$  (S.E.M. 0.080)) than the control groups (plus tilmicosin  $39.82^\circ\text{C}$  (S.E.M. 0.066) and minus antibiotic  $39.88^\circ\text{C}$  (S.E.M. 0.043)) (Fig. 2). However, after the first day of the dust treatment series, significant differences between the mean rectal temperatures of the principals and the controls were not seen.

After a week of rest from dust treatments, a second set of 7-day series of dust treatments was started, and the dust principal groups were not significantly different in mean rectal temperature than the control groups. The trend on the first day of the dust treatment series was for the principal groups to have higher mean rectal

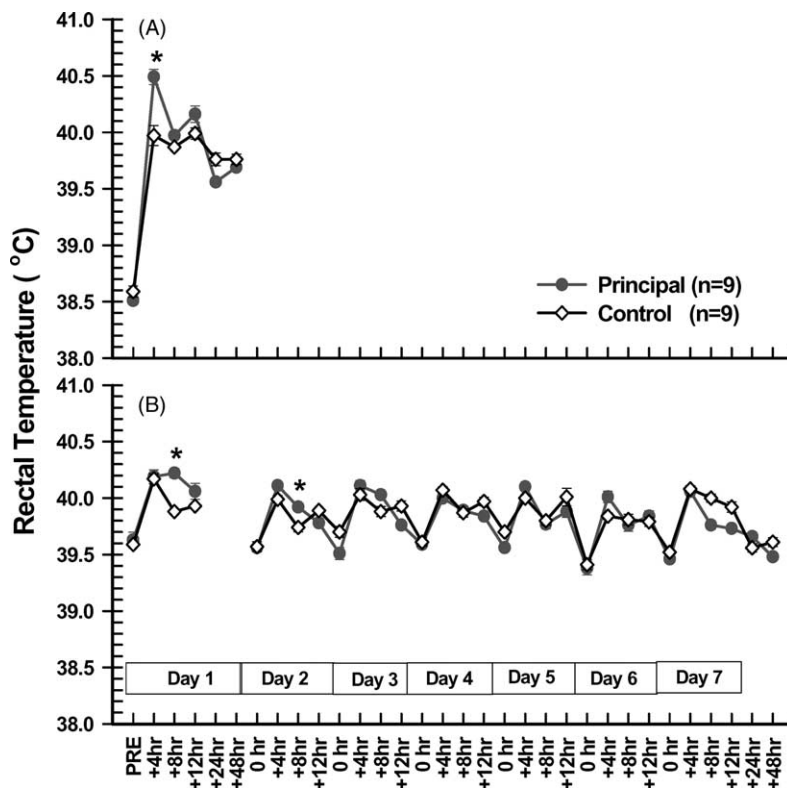


Fig. 2. Mean rectal temperature ( $^\circ\text{C}$ ) of principal dust group without antibiotic after one 4 h dust treatment (A) which was followed by an 18-day rest period, after which a 7-day series of 4 h dust treatments were administered (B). The asterisk indicates a significant rectal temperature increase in the principal dust group compared to the control group without antibiotic.

temperatures (plus tilmicosin 40.12 °C (S.E.M. 0.108) and minus antibiotic 40.10 °C (S.E.M. 0.085)) than the control groups (plus tilmicosin 39.69 °C (S.E.M. 0.051) and minus antibiotic 39.83 °C (S.E.M. 0.120)) at 8 h.

After another week of rest, the third set of 7-day dust treatments was started and the four groups' mean rectal temperatures were significantly ( $P \leq 0.0001$ ) different. On the first day of the dust treatment series, at 8 h, the trend of the principal groups mean rectal temperatures were higher (plus tilmicosin 40.19 °C (S.E.M. 0.098) and minus antibiotic 40.22 °C (S.E.M. 0.127)) than the controls (plus tilmicosin 39.86 °C (S.E.M. 0.070) and minus antibiotic 39.99 °C (S.E.M. 0.070)). Overall tilmicosin did not influence the mean rectal temperature of the four groups.

### 3.8. Clinical effects, clinical pathology, and necropsy results

No panic or restlessness was observed during the 4 h dust treatment to the principal goats. Occasionally some goats would cough during the dust treatment. Coughing in general seemed to increase for 1 to 2 h after the principals were returned to their pens. When the principal goats were removed from the dust tent, their hair coat was very dark with dust particulate matter. The principal groups ( $n = 19$ ) mean rectal temperature significantly increased at 4 h ( $P \leq 0.03$ ) and 8 h ( $P \leq 0.01$ ), following the dust treatment compared to the mean temperature of the controls ( $n = 18$ ). The mean rectal temperature became maximum (40.5 °C, S.E.M. 0.127) 4 h following the dust treatment (Fig. 2). The mean rectal temperatures of the four groups were normal 24 h post-dust treatment. During the 7-day dust treatment series, there was a significant increase ( $P \leq 0.01$ ) in mean rectal temperature of the principals at 4 h post-dust treatment on the first day only, compared to the controls. A state of tolerance appeared to develop after repeated dust treatments (Fig. 2).

The mean total WBC counts can only be compared for the principal dust group without antibiotic and for the control group without antibiotic for the first dust treatment exposure and for the first 7-day series of dust treatments. There was a significant difference ( $P \leq 0.0001$ ) in mean total WBC counts between the control group and the principal group. These differences occurred at 12 h (principal

group, 21,407 cells/mm<sup>3</sup> (S.E.M. 1565) and control group, 15,291 cells/mm<sup>3</sup> (S.E.M. 705)) after the dust treatment on the first day and at 24 h (principal group, 15,147 cells/mm<sup>3</sup> (S.E.M. 777) and control group, 13,811 cells/mm<sup>3</sup> (S.E.M. 676)) after the dust treatment on the seventh day (Fig. 3).

There were no significant differences in fibrinogen concentration between the tilmicosin treated principals and the non-antibiotic treated principals after one dust treatment, and the same relationship was found for the control groups. Therefore, the two groups of principals (18 principals) were combined. The two groups of controls (18 controls) were also combined. There were no significant differences between the controls and principals based on the General Linear Models procedure analysis. Mean control fibrinogen was 320 mg/dl (S.E.M. 16.5) and the mean principal fibrinogen was 330 mg/dl (S.E.M. 14.7). Following one dust treatment, the principals significantly ( $P \leq 0.05$ ) increased to 400 mg/dl (S.E.M. 38) at 4 h, 439 mg/dl (S.E.M. 30) at 8 h, and 461 mg/dl (S.E.M. 37) at 12 h from their 24 h pre-dust base line (mean fibrinogen 289 mg/dl (S.E.M. 26)), based on the Dunnett's *t*-test. This increase was not seen in the controls after one 4 h stay in the tent, nor was it seen in the controls following seven 4 h tent treatments, or in the principals following seven consecutive dust treatments. Very few goats in any group had a measurable haptoglobin response.

The goats were euthanatized with a barbitol solution and the esophagus was immediately ligated to prevent regurgitation. The euthanasia and necropsy of three principal goats with tilmicosin and three principal goats without antibiotic was timed 8 h after the last 4 h dust treatment, in order to have the goats at optimum time, according to their fever response. The remaining 12 principal goats (6 with tilmicosin and 6 without antibiotic) were necropsied 96 h after their last dust treatment. The pathologist did not know which were controls or principals. There were no gross observable differences between the control and principal respiratory tracts. All goats had a mild atelectasis of the right apical lobe of the lung.

The following histologic changes were observed in the six principal goats (three with tilmicosin and three without antibiotic) necropsied 8 h following dust treatment. (1) There was a mild alveolar septal hypercellularity by infiltration of lymphocytes, macrophages and neutrophils. (2) There was moderate multifocal

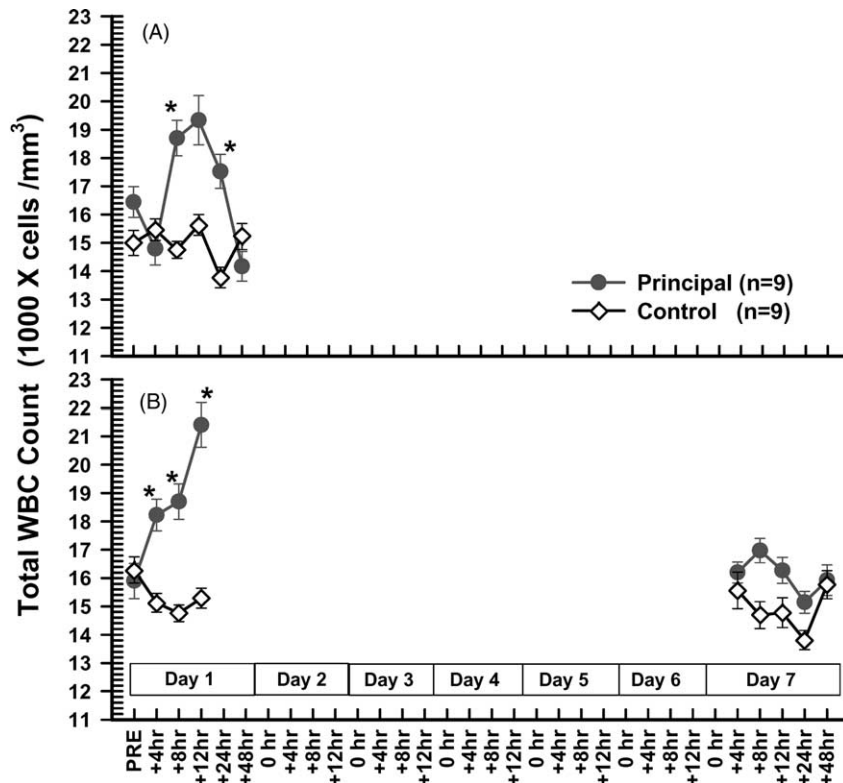


Fig. 3. Mean total white blood cell (WBC) count of principal dust group without antibiotic after one 4 h dust treatment (A) which was followed by an 18-day rest period, after which a 7-day series of 4 h dust treatments were administered (B). The asterisk indicates a significant increase in total WBC count of the principal dust group compared to the control group without antibiotic.

airway exudation of neutrophils and macrophages containing foreign particulate and siliceous material. (3) There was a marked increase in bronchial associated lymphoid tissue compared to controls. The diagnosis was mild acute exudative bronchointerstitial pneumonia. No significant microscopic lesions were observed in the control goats.

The following histological changes were observed in the 12 principal goats (6 with tilmicosin and 6 without antibiotic) necropsied 96 h following dust treatment. (1) There was a marked increase in bronchial associated lymphoid tissue compared to the controls. (2) There was minimal accumulation of macrophages containing foreign particulate and siliceous material in widely scattered airways. The histological diagnosis was increased numbers of pulmonary airway macrophages containing foreign material and increased bronchial associated lymphoid tissue.

Significant gross or microscopic differences were not observed between the two principal treatment groups.

#### 4. Discussion

The long-term results of the nutrition part of the experiment were unexpected in that the twenty-two 4 h dust treatments improved the efficiency of feed utilization. However, in the short term, feed intake was decreased for 4 days in the principal groups following the dust treatment compared to the control groups. The endotoxin in the feedyard dust caused the goat rectal temperatures to increase above 40.6 °C, 4–8 h after endotoxin/dust challenge and a dramatic leukocytosis occurs at 12–24 h. This phenomenon was observed in mature sheep (Purdy et al., 1999,

2002a) and in light-weight market stressed cattle after endotoxin/dust treatments (Purdy et al., unpublished, 2002a,b). Therefore, after a single dust treatment for several days the goats were stressed and it reduced their appetite for several days. It appears that on repeated dust treatments, a state of rectal temperature tolerance occurred. This also occurred in mature sheep, however, they had the ability (after a week of rest) to react to endotoxin at 4–8 h after an aerosol dust treatment by showing a characteristic significant temperature increase compared to controls (Purdy et al., 1999, 2002a).

The tilmicosin antibiotic dust interaction was unexpected. The control goats not exposed to dust nor receiving antibiotic had lower feed intakes and lower weight gains than the principal goats. The antibiotic tilmicosin is used almost exclusively in cattle to treat acute bovine pneumonia (Frank et al., 2000). This disease is caused by a number of physiological and biological stressors, which occur while the animals are in the marketing system (Purdy et al., 1986). However, the two most important Gram-negative bacteria in this complex syndrome are *Mannheimia haemolytica* (previously called *Pasteurella haemolytica*), and *Pasteurella multocida* (Frank and Smith, 1983). A single dose of tilmicosin appears to minimize the stress seen when rabbits are handled (McKay et al., 1996).

According to the insert provided by Elanco Animal Health, a division of Eli Lilly and Co., Indianapolis, IN, Micotil (tilmicosin) has an in vitro antibacterial spectrum that is predominantly Gram-positive with a narrow activity against Gram-negative bacteria, *M. haemolytica*, *P. multocida*, and *Haemophilus somnus* and *Mycoplasma* spp. Goats are also susceptible to these respiratory types of bacteria (Purdy et al., 1990, 1993; Purdy and Foster, 1991; Purdy and Straus, 1995). When goats are market stressed, *M. haemolytica* is frequently isolated from the nasal turbinates, just like that seen in stressed calves (Purdy et al., 1986; Frank et al., 1994, 2002). In cattle the palatine tonsils serve as reservoirs (Frank et al., 1993) for *M. haemolytica* to reside in until marketing and transportation stress causes them to multiply. The goats in this study never exhibited signs of upper respiratory tract infections and their histologic lesions were not those of acute respiratory tract disease. Their fever and leukocytosis episodes were always related to the effects of endotoxin and not infection. Therefore,

we do not think that the positive protective effect of tilmicosin against dust was due to inhibition of *M. haemolytica*. *M. haemolytica* and *Pasteurella* in general are fastidious and die quickly in the environment (Purdy et al., 2001), they are especially susceptible to desiccation, so we have no reason to believe that they were in the dust. The concentration and identification of some Gram positive bacteria, Gram-negative bacteria and fungi contained in the dust taken from the feedyard surface, were reported in detail in this report. Also, we have never isolated *M. haemolytica* or *Pasteurella* from feedyard dust. Very few Gram-negative bacteria survive in aerosolized dust, because of the desiccation.

It is our contention that the protective effect afforded by tilmicosin was due to its effect against the large Gram-positive aerosolized bacterial population and high doses of endotoxin. It has been reported (Lakriez et al., 2002), that tilmicosin reduces lipopolysaccharide induced, macrophage prostaglandin E2 (PGE2) production. This compound is important to the inflammation process. Alveolar macrophages play a major role in developing the inflammation cascade within the lung in response to bacterial products and endotoxin (Yoo et al., 1995).

The organic dust contains natural endotoxin, bacteria and fungi spores attached to different sizes of the organic dust particles, and some of these microbes can also be free of dust particles. But all of these fraction attached to dust particles or free can induce inflammation of the lungs (Purdy et al., 2002a). Tilmicosin accumulates in the lungs of animals with inflammation and/or infections with inflammation which actually improves its penetration (Morck et al., 1997; Modric et al., 1999). In lung lesional tissue (induced by *M. haemolytica*) from the bovine which received tilmicosin, large areas with limited inflammation were observed compared to large areas of inflammation in lesions from non-treated controls (Goubau et al., 2000).

It has been suggested that tilmicosin induces neutrophil apoptosis and that this may have an anti-inflammatory effect (Chin et al., 2000). Endotoxin inhaled by itself induces inflammation of the respiratory tract when it triggers epithelial tissue cells to release chemotactic substances that recruit monocytes in the airways (Koyama et al., 1991). Mononuclear phagocytes have the potential to damage structures

of the airways by releasing a number of substances which modulate airway inflammation. This recruits leukocytes to the site and further adds to the inflammatory process when these cells disintegrate and discharge their lytic enzymes.

Histological verification of a mild alveolar septal hypercellularity by infiltration of lymphocytes, macrophages and neutrophils were observed in our goat study. Moderate multifocal airway exudation of neutrophils and macrophages containing foreign particulate and siliceous material was observed, along with a marked increase in bronchial associated lymphoid tissue compared to controls.

It was reported (Croft et al., 2000), that lambs raised by multiparous ewes were heavier, when the ewes were treated with tilmicosin compared to those treated with a placebo. Also, it is not uncommon for animals to gain more weight when antibiotics are added to the feed compared to non-antibiotic fed animals (Stock et al., 1995; Witte, 1998; Onisade and Babatunde, 1997; Antimicrobial Feed Additives, 1997). The growth promoting effect of antibiotics is very advantageous when animal performance is low (Tomke and Elwinger, 1997).

Humans following repeated endotoxin exposure develop a tolerance which is lost after a few days of non-exposure (Schilling, 1956). This “Monday chest tightness” is accompanied by a reversible decrease in ventilatory function after repeated endotoxin exposure (McKerrow et al., 1958; Merchant et al., 1974). After multiple dust treatments, the goats became refractory (tolerant), but they lost their tolerance after a few days of rest from endotoxin exposure. The reactions observed in the goats following one dust treatment were similar to those observed in humans following exposure to purified endotoxins (Rylander et al., 1989; Clapp et al., 1993). In fact, tolerance to lipopolysaccharide (endotoxin) in human blood monocytes has been explained on the cellular level (Ziegler-Heitbrock et al., 1995).

It is recognized that CD14 on phagocytes is an important receptor site for endotoxins (Morrison et al., 1993; Rousset and Dubreuil, 2000) and that lipopolysaccharide-binding protein forms a complex and interacts with CD14, leading to cellular activation (Tobias and Ulevitch, 1993). Also, soluble CD14 may act as a negative regulator of T-cell activation and function in humans (Nores et al., 1999).

In conclusion, endotoxin/dust induces in goats a rapid and transitory significant increase in rectal temperature at 4–12 h and a leukocytosis at 12–24 h, following a 4 h aerosol dust treatment in a closed tent. The leukocytosis was produced by an absolute increase in neutrophils at 4–8 h, and an absolute decrease in lymphocytes in 4–8 h following dust treatment. The rectal temperature response was not present 24 h after endotoxin/dust exposure and the leukocytosis was not seen 48 h after endotoxin/dust exposure. On daily 4 h repeated endotoxin/dust treatments, the goats became tolerant to the characteristic rectal temperature increase and the characteristic leukocytosis that was present following one single dust treatment. During the 7-day dust treatments, the leukocytosis occurred at 12 h on the first day of the 7-day series only. It appeared that the significant rectal temperature increase and leukocytosis after a single dust treatment was in response to the endotoxin part of the feedyard dust and not due to viable microbes. In this study, we have described acute endotoxin/dust reactions in young goats by following several parameters (rectal temperature response, total WBC counts, absolute neutrophil and lymphocyte counts, fibrinogen and haptoglobin concentrations) following 4 h dust treatments in a closed tent.

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